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The use of intralesional epidermal growth factor in the treatment of diabetic foot ulcers

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ABSTRACT

Aims: The mitogenic and cell protective effects of epidemal growth factor (EGF) in wound healing stimulate the growth, recovering the surface of the wound area. In this research we tried to elucidate the effectiveness of intradermal EGF application on wound healing in diabetic foot ulcers regarding the fact that EGF can accelerate the formation of skin cover layer on the infected surface, even in relatively ischemic cases.

Methods: The data of 68 patients who applied to our institution's orthopedics and wound care outpatient clinic with the diagnosis of diabetic foot ulcer, who underwent wound care, debridement and follow-up were retrospectively analyzed. All of the patients included in this study were classified as Wagner Stage III and Stage IV diabetic foot ulcers and were followed up with standard wound care. EGF application was initiated if there was not enough bleeding on the wound borders and defect floor after debridement.

Results: The rate of patients with 50% or more granulation in the second week of treatment in the groups was 35.7% (n=10) in the standard treatment group, it was 60% (n=24) in the EGF group ($p<0.05$). Complete granulation rates at the fourth week of treatment in patients who did not show complete granulation in the second week of treatment was 30.8% (n=8) in the standard treatment and 61.1% (n=22) in the EGF treatment ($p<0.05$). Similarly, in patients who did not show complete granulation in the fourth week of treatment, complete granulation rates at the sixth week of treatment was found to be 44.4% (n=8) in standard treatment and 85.7% (n=12) in EGF treatment ($p<0.05$).

Conclusion: According to the results of this study, intradermal EGF application in diabetic foot ulcers may positively affect wound healing by accelerating the formation of a skin cover layer.

Keywords: Diabetic foot ulcer, EGF, epidermal growth factor, diabetic ulcer, wound care

INTRODUCTION

World Health Organization (WHO) said that the prevalence of diabetes is expected to rise to 366 million in 2030.[1,2] Diabetic foot ulcer is a significant and devastating diabetes complication that reduces patients' quality of life. The risk of non-traumatic amputation in diabetic patients is 5 to 50 times higher than in non-diabetic patients. For this reason, unless diabetic feet are not treated properly and timely, they can go for amputation.[3]

According to the WHO, the risk of developing diabetic foot ulcers in patients with diabetes is 15%.[2,4] 25% of diabetic patients admitted to the hospital have foot problems.[5] Most diabetic foot amputations (62%) performed are below the knee level.[6] In the first three years following the amputation, re-amputation is required in 30-60% of the cases. Almost half of the (40-55%) amputated patients face the same scenario on the opposite side within 1-5 years. Death in the first three years has been reported in 35-50% of patients undergoing amputation.[7]

EGF was first isolated from mice sub-maxillary glands, and it is commonly found in the salivary glands.[8] EGF stimulates the growth and proliferation of fibroblasts, keratinocytes and vascular endothelial cells involved in forming wounded tissue. EGF binds to epidermal growth factor receptors on the cell surface with great affinity and stimulates the protein-tyrosine kinase activity of the receptor in the cell. This tyrosine kinase activity initiates the signal transduction cascade that causes many biochemical changes within the cell, such as an intracellular calcium level increase, glycolysis and protein synthesis increase, and an increase in the emergence of genes such as the epidermal growth factor receptor (EGFR) gene that causes DNA synthesis and cell growth.[9]

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The mitogenic and cell protective effects of EGF in wound healing stimulate the following mechanisms;-generative cells to the wound site-angiogenesis, accumulation and maturation of the extracellular matrix-like granulation tissue-wound contraction by myofibroblast activation, proliferation and growth-re-covering the surface of the wound area by migration, proliferation and growth of epithelial cells.[10]

In diabetic wounds, the constant presence of neutrophils, macrophages, and related cytokines in the environment predisposes to destruction in the microenvironment, and the balance between matrix synthesis and degradation is disturbed. Growth factors in diabetic wounds decrease due to increased enzymatic degradation in the wound area, thus significantly reducing diabetic wound healing.[11] Increased blood glucose and metabolites are toxic to EGF receptors, fibroblasts and endothelial cells and can be counted as a separate factor that delays wound healing.[12]

Particularly in ischemic-type diabetic foot ulcers, granulation rarely occurs because the proliferation phase is eliminated. Since the ultimate goal in treating such patients is to prevent the extremity from undergoing amputation, healing a wound without arterial blood perfusion emerges as a complex problem with a low chance of success.[13]

The biological activity of EGF occurs by binding to receptor molecules in mesenchymal and epithelial tissue. Fibroblast, endothelial cell-like cell types responsible for wound healing have EGF receptors, and it has been shown that the presence of EGF in the medium stimulates these cells and increases their proliferation.[11]

EGF is used to stimulate the formation of granulation tissue, which is beneficial in the treatment of diabetic foot in stage 3 and 4 Wagner classification of patients with neuropathic and ischemic ulcers in an area larger than 1 cm^2, and thus for secondary healing or closure of the wound with skin autograft with other conventional regimens.[11,14]

The primary aim of this research was to elucidate the effectiveness of intradermal EGF application on wound healing in diabetic foot ulcers regarding the fact that EGF can accelerate the formation of the skin cover layer (closing of the skin defect) on the infected surface, even in relatively more ischemic diabetic foot ulcers.

Additionally, we have tried to investigate and compare the patients who were followed up with wound debridement only in diabetic foot ulcers who had decreased blood supply in the wound area and those who underwent intradermal EGF in addition to debridement and to evaluate the effect of EGF on wound healing.

METHODS

The study was carried out with the permission of Dışkapı Yıldırım Beyazıt Training and Research Hospital Clinical Researches Ethics Committee (Date: 05.04.2021, Decision No: 108/18). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Informed consent was obtained from all participants.

The data of 68 patients who applied to our institution's orthopedics and wound care outpatient clinic between 2018-2020 or were consulted by other departments with the diagnosis of diabetic foot ulcer, who underwent wound care, debridement and follow-up were retrospectively analyzed.

All patients included in this study were classified as Wagner Stage III and Stage IV diabetic foot ulcers (n=68). They were followed up with standard wound care methods and repetitive debridements, and the improvement of blood supply and wound healing was observed. If there were improvement in wound healing then we continued debridemend (n=28). If there was insufficient bleeding on the wound borders and defect floor after debridement, an EGF application was initiated (n=40), usually decided after the first two debridements. The same investigator has performed all EGF application decisions. As a result, debridement was applied to 28 of 68 patients, and debridement with intralesional epidermal EGF was applied to 40 of them.

Intralesional epidermal EGF 75 micrograms (every three days)1 were applied to patients with adequate wound healing. If there was ischemia (no improvement and granulation, insufficient blood supply) after treatment, the patients were evaluated with digital selective angiography (DSA). In patients with severe ischemia, if DSA did not show sufficient blood supply for wound healing, direct amputation was recommended before the patients underwent EGF application. Of the treatment groups, amputation was performed in 21.4% (n=6) of patients who received only debridement and 10% (n=4) of patients who received both debridement and EGF treatment.

Statistical Analysis

Patient data collected within the scope of the study were analyzed with the IBM Statistical Package for the Social Sciences (SPSS) for Windows 23.0 (IBM Corp., Armonk, NY) package program. Frequency and percentage for categorical data and mean and standard deviation for continuous data were given as descriptive values. For comparisons between groups, the "Independent Sample T-test" was used for two groups, and the "Pearson Chi-Square Test" was used to compare categorical variables. The results were considered statistically significant when the p-value was less than 0.05.

RESULTS

Sixty-eight patients with Wagner Stage III and Stage IV diabetic foot ulcers (DFU) were evaluated. The distribution of demographic and clinical findings of the patients included in the evaluation is given in **Table 1**. Among the evaluation groups, 28 patients with relatively good circulation and satisfactory blood flow in classical wound debridement were followed up with standard wound care. In the second group, 40 patients who did not develop granulation with standard wound care, had insufficient blood supply and did not improve with classical wound debridement were followed up with intralesional treatment of 75 human-derived EGFs every three days. Complete granulation was observed in all patients after eight weeks (**Figure 1**). The mean time to complete granulation of the patients was 6.6 weeks in the standard treatment group and 4.9 weeks in the EGF group. This difference was statistically significant (p<0.05). In the first year after recovery, recurrence was seen in 42.9% (n=12) of the standard treatment group and 15% (n=6) of the EGF group (p<0.05).

Figure 1.

There was no statistically significant relationship between the treatment groups and the gender of the patients (p>0.05). While there was a significant difference between the ages of the patients and the treatment groups, it was determined that the mean age of the patients who received standard wound care treatment was lower than that of the group that received EGF treatment (p<0.05).

There was a statistically significant difference between the treatment groups in terms of the mean lesion sizes of the patients (p<0.05). The mean lesion size of the EGF treatment group was higher than the standard treatment group.

While the rate of patients with 50% or more granulation in the second week of treatment in the groups was 35.7% (n=10) in the standard treatment group, it was 60% (n=24) in the EGF group (p<0.05). In the second week of treatment, complete granulation was observed in 7.1% (n=2) of the standard treatment group and 10% (n=4) of the EGF group, but this difference was not statistically significant (p>0.05).

Complete granulation rates at the fourth week of treatment in patients who did not show complete granulation in the second week of treatment were 30.8% (n=8) in the standard treatment and 61.1% (n=22) in the EGF treatment (p<0.05). Similarly, in patients who did not show complete granulation in the fourth week of treatment, complete granulation rates at the sixth week of treatment were found to be 44.4% (n=8) in standard treatment and 85.7% (n=12) in EGF treatment (p<0.05).

Complete granulation was observed at the eighth week in all 12 patients (10 who received standard wound therapy and two who received EGF therapy) without complete granulation at the end of the sixth week.

Of the treatment groups, amputation was performed in 21.4% (n=6) of patients who received standard wound treatment and 10% (n=4) of patients who received EGF treatment. There was no statistically significant relationship between treatment groups and amputation (p>0.05).

Table 1. Distribution of demographic and clinical findings of the treatment group

Characteristics	Total (n=68)	SWC (n=28)	SWC + EGF (n=40)	p value
	n/n (%) or median±SD	n/n (%) or median±SD	n/n (%) or median±SD	
Gender				1.000
Male	44/68 (64.7)	18/28 (64.3)	26/40 (65.0)	
Female	24/68 (35.3)	10/28 (35.7)	14/40 (35.0)	
Age, years	66±6	64±6	68±5	0.004
Lesion size, cm²	10.9±4.7	9.5±3.9	11.9±5	0.041
At least 50% granulation at 2 weeks	34/68 (50.0)	10/28 (35.7)	24/40 (60.0)	0.049
Complete granulation at 2 weeks	6/68 (8.8)	2/28 (7.1)	4/40 (10.0)	1.000
Complete granulation at 4 weeks	30/62 (48.4)	8/26(30.8)	22/36 (61.1)	0.023
Complete granulation at 6 weeks	20/32 (62.5)	8/18 (44.4)	12/14 (85.7)	0.028
Complete granulation at 8 weeks	12/12 (100)	10/10 (100)	2/2 (100)	NA
Time to complete granulation, weeks	5.6±1.8	6.6±1.5	4.9±1.7	<0.001
Recurrence in the first year after recovery	18/68 (26.5)	12/28 (42.9)	6/40 (15.0)	0.013
Amputation	10/68 (14.7)	6/28 (21.4)	4/40 (10.0)	0.297

DISCUSSION

The results of this study show that intradermal application of EGF significantly accelerates wound healing in diabetic foot ulcers compared to isolated debridement and significantly reduces the occurrence of recurrence and the likelihood of amputation. In addition, it can accelerate the formation of epithelial tissue even in more ischemic and infected diabetic foot ulcers.

The healing of the tissue depends on variables such as the age of the diabetic individual, the duration of diabetes, and the location of the lesion.[16,17] Although the patient may apply directly to the hospital with diabetic foot without knowing that he has diabetes, there is usually a relationship between the duration of diabetes and the development of diabetic foot.[18]

Singla et al.[19] compared 20 patients with standard wound dressings or EGF-impregnated bandages. In the study group, 80-90% granulation was observed in the first week, and 35% in the only debridement group. However, there was no statistically significant difference in granulation in the eighth-week results. The duration of hospital stay and wound closure time were found to be significantly shorter in the study group. In the topical use of EGF, the expected effect is more difficult to obtain due to proteases.[19] In our study, complete granulation was observed in all patients after eight weeks. The mean time to complete granulation of the patients was 6.6 weeks in the standard treatment group and 4.9 weeks in the EGF group, with statistical significance. In the first year after recovery, recurrence was seen in 42.9% of the standard treatment group and 15% of the EGF group, reaching statistical significance.

In the study of Fernández-Montequín et al.[20] consisting of 41 patients, group I was treated with 75 µg EGF, and group II was treated with 25 µg EGF. The EGF was administered 25 µg and 75 µg EGF intralesional three times a week. It was reported that the mean lesion area of the patients was more than 20 cm^2, and they were Wagner 3-4 group patients. Considering the response evaluation and granulation follow-up, 73.9% in group 1 and 50% response in group 2 were observed at the end of five weeks. The application was continued, and in the eighth week, 82.6% response was observed in group 1 and 61.1% in group 2. In more than 30% of the wound area, granulation tissue formation was achieved in most patients in both groups from the first week. More than 60% of these patients developed complete granulation after five weeks. These results were achieved despite Wagner grade 3 or 4 ulcers, often more significant than 20 cm^2, with ischemic and high amputation risk.[20] In our study, all of the patients were in the Wagner 3-4 group, and granulation occurred in 75% of patients and complete wound closure was achieved in 70%.

In a randomized, multicenter, placebo-controlled study by Montequin et al.[21] in 149 patients, group I was treated with 75 µg EGF, group II was treated with 25 µg EGF and group III was treated with a placebo. It was reported that the mean lesion area of the patients was more than 20 cm^2 and more than half of them were ischemic and were in the Wagner 3-4 stage. In the second week of the study, 83.1% response in group 1, 70.8% in group 2 and 39.6% in group 3 were observed. The time to recovery was found to be shorter in the high-dose group. EGF has been shown to accelerate healing. Amputation mainly occurs in ischemic patients.1

In another pilot study by Montequin et al.[21] in patients with Wagner Classification Grade 3-4, mean lesion area 16.3 cm^2, 20 patients received 75 µg EGF intralesional therapy three times a week. Complete granulation was seen in 100% of patients. The mean time to granulation was 23.6 days. Complete wound healing was reported in 75% of patients, and the amputation rate was 0%.21 In the study of Valezquez et al.[22] consisting of 32 patients, complete granulation was achieved in 90.62% of patients, amputation in 9.38% of patients, and a total treatment time was 46.5 days. In our research, while the rate of patients with 50% or more granulation in the second week of treatment was 35.7% in the standard treatment group, it was significantly higher (60%) in the EGF group. Complete granulation rates at the fourth week of treatment in patients who did not show complete granulation in the second week of treatment were 30.8% in the standard treatment and 61.1% in the EGF treatment ($p<0.05$). Similarly, in patients who did not show complete granulation in the fourth week of treatment, complete granulation rates at the sixth week of treatment were found to be 44.4% in standard treatment and 85.7% in EGF treatment ($p<0.05$). Complete granulation was observed at the eighth week in all patients, without complete granulation at the end of the sixth week.

In the study of Alos et al.[23] patients with diabetic foot ulcers from 41 hospitals were prospectively treated with 25 or 75 ug EGF three times a week for a maximum of eight weeks. EGF doses were determined by the physician who had diagnosed ulcers according to whether they were ischemic or not. There were 1788 patients, 43% of whom were ischemic, and 1835 diabetic foot ulcers. Complete granulation was seen in 76% of patients. Amputation was required in 12% of patients during treatment. Most of these cases consisted of those with ischemic and Wagner 3-5 group, and 5% relapse was seen. The most common side effects during application were pain, burning, chills, chills and palpitations at the application site.

In the study of Gonzalez-Acosta et al.[24] when intralesional EGF treatment was added to traditional standard care, the amputation rate decreased from 26.7% to 8.3%.[25] Garcia-Herrera et al.[25] have reported 43.1% to 8.1% in a similar design. Silva et al.[26] achieved 50% and 75% granulation responses in the second week after EGF treatment in patients with neuropathic ulcers. In our study, amputation was performed in 21.4% of patients who received standard wound treatment and 10% of patients who received EGF treatment.

In the study of Gomez et al.[27] 34 patients were treated with 75 ug EGF for a maximum of eight weeks. The placebo group and the EGF group were compared regarding the rate of reduction in ulcer size; 12.5 cm² was observed in the EGF group, and 5.2 cm² in the placebo group, and a statistically significant difference was found between the two groups. The difference between these two groups for newly formed epithelium was 3% and 28%.[27] In our study, the mean lesion size of the EGF treatment group was higher than the standard treatment group, with a statistically significant difference.

In the pilot study of twenty-nine patients by Acosta et al.[28] wounds over 20 cm² that were previously treated and did not heal were removed. EGF was applied to the patients three times a week, and the predicted amputation was prevented in 58.6% of patients who had an 80% granulation response in the eighth week.[28] In the histological examination of these patients, transformation, granulation and angiogenesis were observed in the matrix tissue. The quality of this healing tissue may be the reason for the decrease in recurrences in follow-up.

The current study had several limitations, primarily the retrospective design and the low number of patients. The general conditions of the patients were not standardized. Therefore, there was no randomization in patient selection. On the other hand, all the procedures were performed same senior surgeon with a standard protocol in place. The patients' DFUs were in different locations, and we could not establish a standard. The intralesional injection of EGF to the exact location might not be correct.

CONCLUSION

Regarding the results of this study, one could state that the effectiveness of intradermal EGF application on wound healing in diabetic foot ulcers has been elaborated. Intradermal EGF application significantly accelerates wound healing in diabetic foot ulcers and significantly reduces the occurrence of recurrence and the likelihood of amputation. Furthermore, EGF can accelerate the formation of a skin on the infected surface, even in relatively more ischemic diabetic foot ulcers.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Dışkapı Yıldırım Beyazıt Training and Research Hospital Clinical Researches Ethics Committee (Date: 05.04.2021, Decision No: 108/18).

Informed Consent: Written informed consent form was obtained from all participants.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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